SUPPRESSION OF PYRUVATE CARBOXYLASE BY GLUCOSE IN THE PERFUSED RAT LIVER

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1. Introduction

The hepatic levels of three of the key enzymes of gluconeogenesis, pyruvate carboxylase (E.C. 6.4.1.1), phosphoenolpyruvate carboxykinase (E.C. 4.1.1.32) and glucose-6-phosphatase (E.C. 3.1.3.9), rise in fasting and conversely decrease on refeeding [1–5], the fourth enzyme, fructose-1,6-diphosphatase (E.C. 3.1.3.11), decreasing during an overnight fast [6–7]. The levels of these enzymes are also elevated in diabetes and lowered on treatment with insulin [8–12]. It is thus likely that insulin rather than food *per se* is the suppressor of the enzyme levels in the fed state, the glucose intake stimulating insulin secretion. If this is so, it is not to be expected that addition of glucose would influence the levels of these enzymes in the perfused liver. This appears to be the case for three of the four enzymes, but we find that the activity of pyruvate carboxylase does decline under such conditions.

2. Methods

Livers from fasting male rats 150 g body weight were perfused with a medium consisting of 80% fresh rat blood from fasting donors diluted with 0.9% saline and heparin at 100 units per ml: total volume of the perfusate was 100 ml, haematocrit 35%. Liver donors were anaesthetised with ether and the liver cannulated *in situ* as described by Miller et al. [13], the whole operation lasted approximately 15 min, the liver being out of circulation 1–2 min. The perfusion apparatus used was a recycling system based on that of Mortimore and Tietze [14]. The stated amounts of glucose were added to the perfusate prior to the cannulation of the liver. After perfusion for four hr at 37° livers were washed through with ice-cold saline, frozen at −80°, weighed (final weight 5–6 g) and stored at −30°.

All enzymes were assayed at 30°: pyruvate carboxylase was assayed in extracts of acetone powders of liver, prepared immediately after termination of the perfusion, by following the incorporation of ^{14}C-bicarbonate into citrate as described by Felicioli and Rossi [15]. Phosphoenolpyruvate carboxykinase was assayed by the method of Foster et al. [16] using 6 mM $MgSO_4$ and 0.1 mM $MnCl_2$ as the activating ions, the phosphoenolpyruvate generated being measured by the method of Lohmann and Meyerhof [17]. Fructose-1,6-diphosphatase was followed spectrophotometrically by the method of Underwood and Newsholme [18], using 5 mM $MgSO_4$ and 0.05 mM fructose-1,6-diphosphate for maximal activity. Glucose-6-phosphatase was assayed at pH 6.5 in 40 mM cacodylate buffer by measuring the liberation of P_i [19, 20]. Perfusate glucose, liver glycogen, lactate, K^+ and DNA were also estimated [21–25].

3. Results and discussion

Table 1 compares the hepatic content and concentration of the four enzymes from the livers of fed animals and animals after an overnight fast. Since the weight of the liver declines on fasting, the contents show relatively smaller changes than the concentrations. Glucose-6-phosphatase and phosphoenol-

Table 1
Activities of the hepatic gluconeogenic enzymes from fed and fasted rats.

	Fed rats		Fasted rats	
	(μmoles/g/min)	(μmoles/100 μg DNA-P_i/min)	(μmoles/g/min)	(μmoles/100 μg DNA-P_i/min)
Glucose-6-phosphatase	6.40 ± 0.60	3.70 ± 0.35	16.50 ± 0.55	5.40 ± 0.40
Fructose-1,6-diphosphatase	7.00 ± 0.60	4.00 ± 0.40	9.10 ± 0.23	3.00 ± 0.15
Phosphoenolpyruvate carboxykinase	3.60 ± 0.30	2.05 ± 0.10	10.10 ± 0.25	3.30 ± 0.19
Pyruvate carboxylase	3.30 ± 0.35	1.90 ± 0.23*	7.35 ± 0.45	2.40 ± 0.19*

Each figure is the mean ± S.E. of the mean of four observations for fed rats and 8 for fasted. Significance of the difference (p) due to fasting was < 0.05 except for *.

Table 2
Effect of increased perfusate glucose concentration on the activities of the enzymes of gluconeogenesis in livers from fasted donors.

	No additions to perfusate		250 mg% glucose in perfusate	
	(μmoles/g/min)	(μmoles/100 μg DNA-P_i/min)	(μmoles/g/min)	(μmoles/100 μg DNA-P_i/min)
Glucose-6-phosphatase	17.80 ± 0.63	6.60 ± 0.40	19.13 ± 0.77	5.80 ± 0.43
Fructose-1,6-diphosphatase	9.45 ± 0.60	3.30 ± 0.29	9.60 ± 0.30	3.13 ± 0.25
Phosphoenolpyruvate carboxykinase	9.53 ± 0.40	3.30 ± 0.21	9.33 ± 0.31	3.05 ± 0.23
Pyruvate carboxylase	6.77 ± 0.22	2.33 ± 0.06	4.51 ± 0.20*	1.46 ± 0.03*

Each figure is the mean ± S.E. of the mean for four observations.
* $p < 0.001$; in all other cases there was no significant difference between the two conditions.

pyruvate carboxykinase show the largest percentage rises, with a smaller rise in pyruvate carboxylase concentration, while the content of fructose-1,6-diphosphatase is significantly lower after an overnight fast.

Table 2 compares the content and concentration of these four enzymes in livers from fasted animals after perfusion for four hours either with perfusate to which no additions were made or with medium with 250 mg glucose added per 100 ml. There is no significant change in the measured activities of glucose-6-phosphatase, fructose-1,6-diphosphatase or phosphoenolpyruvate carboxykinase during perfusion with or without added glucose, but a 40% fall in that of pyruvate carboxylase was found for perfusions in the presence of the sugar. Glycogen was deposited in these latter perfusions, 3.03 ± 0.29 mg/g, as opposed to 0.92 ± 0.13 mg/g and 0.92 ± 0.19 mg/g in fasted and fasted livers perfused without substrate respectively. Twice as much lactate was present in livers perfused with added glucose (6.6 ± 0.67 μmole/g compared with 3.03 ± 0.02 μmole/g). After allowing for glucose consumption by red cells in the perfusate (0.6 μmole per min per 100 ml of medium when circulated through the apparatus), output of glucose by the liver in the absence of added sugar was about 0.3 μmole per g per min, whereas in its presence there was uptake of about 0.1 μmole per g. Hepatic potassium concentration was similar in all perfusions and averaged about 97 μmole per g.

The available evidence for the induction of enzymes such as glucokinase and glycogen synthetase with feeding strongly suggests that insulin rather than glucose is the primary inducer. Similarly, Weber et al. [26] found that the levels of the gluconeogenic

enzymes glucose-6-phosphatase and fructose-1,6-diphosphatase in long fasted rats showed no response to injection of large amounts of glucose unless insulin was given at the same time (a nice demonstration of the importance of intestinal hormones in the control of insulin release in response to glucose [27]). Catabolite repression, although widespread in bacterial systems [28, 29], has to date been reported only for two mammalian enzymes, serine dehydratase and ornithine transaminase [30], where activity induced by a high protein diet is repressed more effectively by glucose than by insulin. Pyruvate carboxylase, by its response to glucose in the absence of added hormones in the perfusion system, offers another example of this mechanism in mammalian liver. The perfused organ constitutes a particularly favourable preparation in which to observe the effect of glucose unrelated to the release of hormones. Since pyruvate carboxylase is predominantly a mitochondrial enzyme the mechanism of the glucose effect is not clear. Glucose up to 10 mM added to the assay system is without effect. Though the hepatic content of pyruvate carboxylase is usually found to rise in diabetes [10, 31, 32] some authors have found little change [33, 34]. It is possible that in such instances glucose suppression has occurred *in vivo*. It may also explain the inhibition of glucose synthesis from alanine in livers perfused with glucose as shown by Rundermann and Herrera [35].

A recent report [36] demonstrates an effect of glucose on the perfused liver which converts glycogen synthetase to the active form and phosphorylase to the inactive form possibly through activation of phosphorylase phosphatase [37]. It could be that our results are indicative of different forms of pyruvate carboxylase subject to interconversion in response to glucose.

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